Sida cordifolia Seed Phytochemical Evaluation as an Antioxidant and Antimicrobial

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Abstract:

Medicinal plants contain clusters of bioactive compounds like alkaloids, flavonoids, tannins, etc. which are used against different degenerative ailments. *Sida cordifolia* has been prescribed in male infertility problems in the local communities. In this study, the ethanolic extract of *Sida cordifolia seeds* was prepared by microwave-assisted extraction (MAE) process. *Sida cordifolia* extract was qualitatively tested for different phytochemicals and the total phenolic content of the extract was calculated in terms of gallic acid equivalent (GAE) per gram of dry extract while the content of flavonoids was determined as quercetin equivalent. The IC50 value were 10.29 and 33.36 in DPPH and FRAP assay respectively. Antioxidant enzymes SOD, CAT, and POD were also measured as 19.46, 38.94, and 4.75 units/mg respectively. At 1000mg/ml it also possesses 13.32 % thrombolytic activity against standard indicative of enhancing blood perfusion to tissues. Antimicrobial activity of *Sida cordifolia* (Riaz-1) against gram-positive and gram-negative strains of bacteria and fungus was also detected against standards. The study results have created an opportunity for researchers and clinical practitioners to treat oxidative stress-related diseases by natural drugs.

Key Word: Antimicrobial, Antioxidants, Infertility, Phytochemical, Reactive oxygen species, *Sida cordifolia*, Thrombolytic

Introduction.

According to the updated survey reported by WHO, nearly above 60-80% people of in developing countries directly or indirectly depend on plants and plant-based products to resolve their health issues [1]. From beginning of the world, these medicines are used against fever, antimicrobial, hepatoprotective, antioxidants and chronic disorders like diabetes or cancers etc. In many developing countries alternative medicine has gained popularity and used as prime component of primary healthcare system. Medicinal plants contain clusters of bioactive compounds like alkaloids, flavonoids, tannins etc. which are used against different degenerative ailments [2]. Plants curative potential extensively exploited in herbal medicine and approximately 25% of prescribed drugs around the globe are plant origin ([3] [4]).

Sida cordifolia (Family: Malvaceae) is an annual shrub growing wildly in Pakistan and favorably grow in damp climates [5]. The different parts of plants such as seeds, leaves, and roots are commonly used as medicine in the Indopak [6]. *S.cordifolia* contains many compounds like alkaloids, saponins , phenols and flavonoides with multiple pharmacologic activities [7]. Traditionally, it has been extensively used as anti-inflammatory, antioxidants, analgesic, anti-inflammatory, rheumatism and improve nervous disability. *Sida cordifolia* also prescribed in male infertility problems in the local communities [1, 8].

There is a very high rate of infertility, 21% representing about a fifth part of the population documented in Pakistan [9]. A large amount of evidences propose that damage by ROS to spermatozoa is the major contributing factor in the development of 30-80% cases of infertility in men [10]. These have destructive effects on spermatozoa's functional and structural integrity, leading to functional loss. The most common cause of sperm damage is the oxidative stress induced by toxicants [8].

Synthetic antioxidants are extensively used in biological systems to work against oxidation despite harmful effects on health. To resolve this global health issue, it's a need of time to a paradigm shift toward nature. Plants and plants-based products can play a pivotal role to cope with this challenge [11]. Various damaging effects caused by ROS are membrane lipid peroxidation, moreover, peroxidation products and their metabolites are highly reactive [12]. They react with biological substances, DNA, amines, and proteins. Recent inquiries show that oxidant scavenging activities of different plants may be linked with defense against stress because of oxidants and different human disorders produced by ROS [13]. Antioxidants may interfere with the process of oxidation through reaction with free radicals, chelation of free catalytic metal and scavenging oxygen [14].

The Indo-Pak subcontinent has a widely rich heritage in the use of therapeutic plants in clinical practice since the earliest times [15]. Moreover, other countries like China, Africa, the USA and Brazil are also used to cure different diseases for centuries [16]. World widely physicians prescribe medicinal plants for different purposes and particularly in rural areas for clinical treatment [17].

Using modern tools such as phytochemical, biochemical, biotechnological and bioinformatics essential bioactive compounds are isolated from medicinal plants for evaluation of their therapeutic activities [18]. Since the 19th century, many reported bioactive chemical constituents are being used as active ingredients in present modern medicine [19]. Medicinal formulation phytochemicals are classified as secondary and primary metabolites. Proteins, lipids, chlorophyll, and carbohydrates are classified as primary metabolites while bioactive secondary metabolites are steroids, flavonoids, terpenes, alkaloids, phenolic compounds, and coumarins for therapeutic purposes [20].

The natural antioxidants present in plants like alkaloids, flavonoids, and phenols play a vital role in the healthcare system [21]. Phenolic acids, flavonoids, bioflavonoids, anthocyanins, and isoflavonoids are subclasses of phenols having properties of antioxidants and work against infectious diseases, allergies, ulcers, tumors, platelet aggregation, reproductive issues, cardiovascular diseases and can reduce cancer risk [22]. Currently, research on plants has been used worldwide and is important in traditional systems [23]. Antibiotics are the known magic drugs that are playing a vital role against bacterial infections [24]. But due to miss use of these antibiotics leads to less effectiveness and ultimately globally health benefits are under threat in last few decades. Emergence of antibiotics resistance is a worldwide threat, it is much important to search for innovative alternative medicine [25]. Plants or plants originated products due to their temperamental based action and less resistance may be a suitable alternate of these synthetic drugs in future and play pivotal role in treating infectious diseases [26].

Currently, practitioners are using hormones or analogies to treat fertility-related issues which are linked with the feedback suppression of the normal release of hormones having a role in reproduction leading to more serious issues later on. That's why this research study was planned to explore the phytochemicals analysis along with the antioxidant and the antimicrobial profile of *Sida cordifolia* extract to have a dual benefit by avoiding microbial infections having a link with male infertility through antioxidant properties [27].

Materials and Methods

Collection, Authenticity, and Processing of Plant Material

From the local market of Faisalabad, the seeds of *Sida cordifolia* were purchased and authenticated by the Department of Botany, Govt. College University, Faisalabad, under voucher No. GBM-235/21.

Extraction of Plant Material

The seeds were thoroughly examined for contamination and adulteration. After sieving and sifting, the *Sida cordifolia* seeds were mechanically crushed to a moderately coarse powder [28]. The triturated seeds of the crude drug were then extracted with ethanol by microwave assisted extraction (MAE) process[29]. A suitable amount of the drug was soaked in a 500ml glass beaker for a day. It was heated in a microwave oven for 6 minutes at low power and allowed to cool with stirring. The process was repeated 5 times and after that filtered through the Whatman filter paper. Ethanol was recovered under reduced pressure in a rotary evaporator. The thickened extract was further evaporated in a water bath to get semi-solid extract which was kept in a refrigerator for further use in experiments.

Inclusion and Exclusion Criteria

The fresh seeds of the latest harvest were included in the study which was confirmed by purchase invoice of vendor and source documents. The old seeds, contaminated, dusty, decomposed and unknown sourced material was excluded from the study. The bacterial and fungal laboratory strains were included in the study which were properly identified, isolated and authenticated with documents. The unknown and clinical isolates were excluded from the study.

Phytochemical Evaluation.

First, the stock solution was prepared by dissolving half gram of ethanolic *Sida cordifolia* extract in twenty mL of methyl alcohol and used for the initial detection and determination of phytochemicals.

Qualitative Evaluation of Phytochemicals.

Sida cordifolia extract was Qualitatively tested for tannins, saponins, flavonoids, steroids, alkaloids and glycosides, etc. tests were performed following the standard protocols coated by Fischer *et al.,* 2012 [30].

Quantitative Evaluation of Phytochemicals.

Determination of Total Phenolic Contents (TPC)

One hundred microliter sample extract was mixed with 0.5 milliliters of folin–Ciocalteu reagent that had been previously diluted with 7 milliliters of deionized water to determine the

total phenolic contents. The solution was allowed to stand for 3min at 25ºC, and 0.2ml of a saturated sodium carbonate solution was added. The mixture was allowed to stand for another 120 minutes in a dark place for the completion of the reaction and formation of complexes. The absorbance of all aliquots was measured at 725 nm. The calibration curve was compared to a gallic acid standard. The total phenolic content of the extract was determined using the gallic acid equivalent (GAE) per gram of dry extract using the following equation for calculation.

$y = mx + b,$ $x = \frac{(y-b)}{m},$ $c = x\left(\frac{v}{m}\right)$

Were,

v = Volume of sample

m = Concentration of sample in g

y = Absorbance of the sample at 725nm

M = Slope

b = Intercept

x = concentration of gallic acid in µg/mL

c = Total Phenolic contents

Total phenolic content was expressed as mg gallic acid equivalents per gram of dry extract of plant samples.

Determination of total flavonoid contents (TFC)

The total flavonoid content was estimated by the aluminum chloride colorimetric method. The number of flavonoids was determined to be equivalent to quercetin. One ml of plant extract was mixed with 2 ml of AlCl3 (2% w/v) in the appropriate solvent (stock solution SS), and the solution was made up to 25 ml with a methanolic solution of acetic acid (0.5% v/v) (Probe solution PS). A methanolic solution of acetic acid (contrast solution CS) was used to make 25 ml of SS from 1 ml. After 30 minutes, the absorbances of PS and CS were measured at 420 nm. The result was mentioned as mg Quercetin Equivalent/ gram dry extract.

Determination of Total Soluble Proteins (TSP)

Following Bradford's [31] instructions, the total protein content in 0.1 g of fresh *Sida cordifolia* seeds was determined by homogenizing the sample in 2mL of phosphate buffer saline (pH 7.2) and centrifuging it for 10 minutes at 16128 g (MIKRO-200 R; Hettich GmbH and Co. KG). For thirty minutes, the sample was kept at room temperature for incubation. A UV-VIS spectrophotometer (Hitachi U-2910, Tokyo, Japan) was used to estimate the mixture's optical density (OD) at 595 nm, and BSA served as the standard.

Evaluation of Anti-Oxidant Activity

DPPH radical scavenging activity

Utilizing the free radical generator DPPH (2, 2-diphenyl-1-picrylhydrazyl) in a manner that was comparable to that of Brand-Williams, Cuvelier, & Berset, 1956, the free radical scavenging effect was ascertained. 1 ml of this solution was added to various plant extract concentrations (6.25, 12.5, 25, 50, 100, and 200 g/mL) to 3 ml of the solution of all extracts in methanol. The DPPH solution contained 0.1 mg. The test extract was then diluted in a 1:3 ratio with the standard DPPH solution. For 90 minutes, the mixtures were kept at room temperature in the dark. Using a UV-VIS spectrophotometer (Genesys 10S UV, Thermo Electron Corporation), the absorbance was measured at 517 nm. The reference was taken from ascorbic acid. The lower absorbance values of the reaction mixture indicated higher free radical scavenging activity of the test. The ability to scavenge the DPPH radical was calculated by using a formula while IC50 was calculated with the standard curve equation given below.

$$RSA\ \%age = \frac{(Ac-As)}{Ac} \times 100 \qquad Ic50 = \frac{(y-b)}{M}$$

where,

The absorbance of the control reaction is called Ac, and the absorbance of all extract samples when present is called As. y denotes the concentration of the sample, b denotes the standard curve's intercept, and M denotes the standard curve's slope. After that each test was carried out in triplicate, and the outcomes were averaged.

Antioxidant activity of radical cations (ABTS)

The ABTS assay was performed using a slightly modified version of the method that Huang described [32]. When 7.45 mM potassium persulfate was mixed with the ABTS solution, ABTS+ cations were formed. Before using the mixture was diluted with ethanol until it shows an absorbance of 0.70+0.02 at 734 nm. after being in the dark for 11 to 15 hours at room temperature. The Genesys 10S UV-VIS (Thermo Scientific) was used to quantify scavenging activity at 734nm absorbance precisely six minutes after adding 100 mL of the sample or the Trolox standard to 3.9 mL of diluted ABTS + solution. The IC50 was calculated using the standard curve equation of sample concentration and RSA percentage, and the results were expressed as (TEAC) Trolox equivalent antioxidant capacity. The calculations were made by using the following formula

$$RSA\ \%age = \frac{(Ac-As)}{Ac} \times 100 \qquad Ic50 = \frac{(y-b)}{M}$$

Where Ac is the absorbance of the standard and As is the absorbance of the sample extract, results were averaged after all tests were performed in triplicate.

Ferric reducing activity power of plant extract (FRAP).

The power of reducing the extract was determined using the method developed by [33]. One mL of a solution containing 10, 20, 30 & 40 µg/mL of extract was mixed with phosphate buffer (2.5ml, 2M, pH 6.6) and potassium ferricyanide (2.5ml, 1%). The mixture was incubated for twenty minutes at 50ºC. Moreover, 2.5ml of trichloroacetic acid (TCA, 10%) was supplemented to the mixture and centrifuged at 1500 rpm for ten min. Upper layer of the solution (2.5 ml) was mixed with FeCl3 (0.5 ml, 0.1 percent) and distilled water (2.5 ml) and the absorbance was estimated to be 700 nm. Increased absorbance shows the increased reducing power of the reaction mixture. The result was presented as RSA % age while IC50 was calculated by using the standard curve equation of sample concentration and RSA % age. The calculations were made by using the formula given as below

$$RSA\ \%age = \frac{(Ac-As)}{Ac} \times 100 \qquad Ic50 = \frac{(y-b)}{M}$$

Where As is the absorbance of the extract samples & reference and Ac is the absorbance of the control reaction and. Results were averaged after taking in triplicate.

Antioxidant enzymes assay.

Fresh *Sida cordifolia* seeds were grinded in a mortar and pestle in the presence of cooled phosphate buffer (50 mM;) for antioxidant enzyme extraction. dithiothreitol (1 mM) and pH-7.0. The supernatant from the centrifugation of this solution at 25200 rpm for approximately 20 minutes at 4 O C was used to measure the antioxidant and enzyme activities following method quoted by [34].

Superoxidase dismutase (SOD) contents.

With minor modifications, SOD activity of *Sida cordifolia* seeds was determined by the method developed by Gong et al. as coated by [35]. For 15 minutes at 78 µmol m/2/s, the glass vials containing the reaction mixture were illuminated with 15 watts of fluorescent light and at 560 nm, the absorbance was measured.

Catalase (CAT), peroxidase (POD) contents.

By Cakmakk et al.'s method [36], minor modifications of the CAT and POD activities were performed. Every 20 seconds, the absorbance of the reaction blend was estimated at 240 nm. Following the breakdown of H_2O_2, the absorbances of the CAT and POD reaction solutions decreased at 420 and 470 nm, respectively. Units of enzyme activity were expressed as mg-1 of protein (U = 1 mM of H_2O_2 reduction min-1 mg-1 protein).

Thrombolytic activity

The technique created by Prasad with minor modifications was used to analyze the extract's thrombolytic activity [37]. Healthy volunteers who have never before used oral contraceptives or anticoagulant treatment had their blood taken. For this experiment, healthy volunteers (n = 3) provided 7 ml of venous blood, which was then transferred to several sterilized, preweighed, 1 ml microcentrifuge tubes. The microcentrifuge tubes were then incubated for 45 minutes at 37 °C. After the clots had formed, serum was completely withdrawn from the tubes, and each one was weighed again to calculate the clot weight

$$\text{Clot wt.} = Clot\ tube\ wt. - Empty\ tube\ wt.$$

Each tube of the microcentrifuge carrying a clot has a complete label on it. Methanol was added to the plant extract, which was then forcefully shaken on a vortex mixer. The test samples were then prepared at different strengths (10, 8, 6, 4, and 2 mg/ml, respectively). The suspension was then added, mixed at night to get rid of the soluble supernatant, and filtered using a 0.22-micron syringe filter. Each labeled (10-2 mg/mL) tube receives 100µl of extract after filtration. Streptokinase and distilled water were both added in equal amounts to the tubes holding the clots that served as positive and negative controls for thrombolytic activity, respectively, at the same time. After that, the tubes were incubated at 37 degrees for 90 minutes and checked for clot lysis [38].

Antimicrobial Studies

Test Organisms

The test organisms were gifted from the Department of Zoology, Faculty of Life Sciences, Government College University Faisalabad. The microorganisms were standard laboratory strains of *Bacillus cereus* and *Staphylococcus aureus* gram-positive*, Escherichia coli* and *Pseudomonas aeruginosa* gram-negative bacteria, and a fungal strain C*andida albicans*.

Susceptibility Test

The antibacterial susceptibility test was conducted using the agar diffusion method described by Garrod *et al.,* 1963 [38]. The zone of inhibition was observed and recorded in millimeters using a metric rule.

Statistical Analysis

All the procedures were performed in triplicate. Shapiro Wilk test was applied to determine the normality of data. The normality p value was insignificant, so the null hypothesis was rejected, the relationship was confirmed, and results were calculated by using independent sample t-test.

Results:

Percentage Yield and Qualitative Phytochemical Screening

The percentage yield of ethanol extract is 9.81 for *Sida cordifolia*. The results of the extraction revealed that *Sida cordifolia* seeds consist of nonpolar and polar compounds.

Alkaloids, tannins, saponins, cardiac glycosides, steroids, and flavonoids were found in the ethanol extract during the phytochemical analysis as shown in Table-1. The phytochemical compound's positive intensity was determined by an arbitrary scoring system of 5 to 1, with 1 representing the lowest possible concentration.

Total phenolic content

The standard curve was drawn with the concentration and absorbance of gallic acid to calculate the total phenolic contents of *Sida cordifolia* as gallic acid equivalent as shown in Figure 1 and the TPC is mentioned in Table 2. TPC was found at 15.76 ± 0.38 mg GAE/g dry extract of the sample.

Total flavonoids

The standard curve equation in Figure 2 was used to convert the *Sida cordifolia* seed's total flavonoids (TF) into quercetin equivalent, and Table 2 lists the TF values.8.71 ± 0.65 mg QE/g dry extract was found in TF.

Total Soluble Proteins

The total soluble proteins (TSPs) level was 1.13 ± 0.05. An earlier study reported the pollen of *Sida cordifolia* to possess (1.4 ± 0.8) protein, whereas, in the present study TSP is not found higher as compared to the fresh flowers reported earlier.

DPPH radical scavenging assay

DPPH is frequently used to measure how well *Sida cordifolia* neutralizes free radicals when assessing a substance's ability to do so. DPPH was utilized as a free radical-generating reagent. At 517 nm, antioxidants from *Sida cordifolia* were able to transform DPPH into yellow diphenyl-picryl hydrazine. As the outcomes, the IC50 value and RSA percentage have been calculated. Table 2 compares the results of the DPPH radical scavenging test with the ethanolic and standard *Sida cordifolia* extracts [39].

Ascorbic acid served as a control so that the standard curve could be plotted as it is determined in figure 03 and figure 04. The IC50 of the alcoholic extract of *Sida cordifolia* was 10.29 while RSA was found 90±0.27 (mg/ml) at 50g concentration.

ABTS radical scavenging assay (TEAC assay)

Sodium persulphate converts ABTS to cationic radical which is bluish in color and absorbance was taken at 734nm. Most of the antioxidants are reactive to the ABTS radical cation. The bluish ABTS cation radical is converted to back during this reaction. The (TEAC) Trolox equivalent antioxidant capacity assay is the name of this test. The ABTS extremist rummaging consequences of the ethanolic concentrate of *Sida cordifolia* in correlation with the norm (Trolox) have been determined in Figure 05.

The standard curve was plotted using the inhibition percentage against various strengths of the research drug's ethanolic extract and Trolox as the standard. This curve (g/ml) showed the ethanolic extract of *Sida cordifolia* had an IC50 of 22.50 while RSA percentage was 94.74±0.21 as shown in Figure 06. It also showed that the inhibition percentage increased with the concentration of the research plant extract in the assay mixture.

FRAP assay

The test solution's color has been changed from yellow to a variety of green and blue hues based on each compound's reducing power. The development of pearl Prussian blue at 700 nm, which indicates a higher reducing power is indicated by higher, was caused by the presence of antioxidants, which caused the conversion of the ferric (Fe+3) form to the ferrous (Fe+2) form. Table 2 displays the comparison of the FRAP test results for the alcoholic extracts to the standard (ascorbic acid) at 700 nm.

The standard curve for calculating the IC50 (g/ml) of each sample was determined using the %age of inhibition against each concentration of the *Sida cordifolia* extract. Additionally, it was found that as the concentration of the study plant extract in the test mixture increased, so did the inhibition rate. The IC50 of the alcoholic extract of *Sida cordifolia* was 33.36 ± 0.49 represented in Figure 07 and 08.

The IC50 values as well as the standard deviation obtained for the *Sida cordifolia* extract using the different techniques discussed above are presented in Table 2. The ethanol extract of *Sida cordifolia* shows activity with higher antioxidant levels than commonly used fertility drugs.

Antioxidant enzymes (CAT, POD, SOD)

The total soluble proteins (TSPs) level was found 1.13 with SEM ± 0.08 mg/g FW in the seeds of *Sida cordifolia*. The CAT contents were found 38.94 with SEM ± 0.08 units/mg as presented; The POD is used as a usual skin caring component in cosmetic products to remove the H_2O_2 from the tissues. The POD Enzymatic antioxidant was found 4.75 ± 0.03 units/mg which is the highest as found in the other drugs used for fertility.

SOD contents are also known as the antioxidant defense in the body. Because they reduce oxidative stress the cause for diseases like heart attack, atherosclerosis, various age-related disorders, stroke and acute as well as chronic inflammatory conditions. The SOD concentration was found 19.46 with SEM ± 0.04 units/mg as shown in Figure 09.

Thrombolytic activity

As a functional indicator of thrombolytic activity, the percentage of clot weight reduction following administration of *Sida cordifolia* at different concentration solutions was used. The traditional usage of *Sida cordifolia* in treating numerous ailments is supported by the fact that the plant ethanolic extract demonstrated substantial thrombolytic action. According to the findings, the ethanolic extract of *Sida cordifolia* (at a dose of 1000 mg/ml) exhibited thrombolytic activity (13.32%). It was shown in Figure 10 that by increasing the amount of dose, the thrombolytic percentage was raised.

Which is linked with better perfusion of gonadal tissue along with generalized body tissue. This speculates it is helpful in the proper nourishment and maturation of spermatozoa. Moreover, it may help in the fluidity and motility of spermatozoa by preventing clumping/clotting seminal fluid.

Antimicrobial Activity:

Antibacterial activity

The antibacterial activity of the selected plant was measured by the diameter (mm) of the zones of inhibition in the selected bacterial colonies. Gentamicin is used as a standard drug. The zone of inhibition of *Sida cordifolia* (Riaz-1) against gram +ve and gram -ve bacterial strains at 40 mg/mL concentrations was observed and the results are demonstrated in Table 3 and Figure 11.

Sida cordifolia has 17mm, 20mm, 18mm, and 17mm ZOI in *S. aureus, B. cereus*, *E. coli,* and *P. aeruginosa,* respectively. While the standard drug gentamicin at 10µg concentration ZOI is shown in the Table against each bacterial strain. Our data express that the ethanolic extract of *Sida cordifolia* showed good antibacterial results as authenticated by standard.

Antifungal activity

Candida albicans fungal strain were used to check the antifungal activity of plant extract against ketocanazole standard drug. The zone of inhibition of *Sida cordifolia* (Riaz-1) against *Candida albicans* was detected, as indicated in Table 3 and figure 12. Ethanolic extract of *Sida cordifolia* produced a 15mm zone of inhibition at 40mg/ml concentration while the standard drug produced 25mm ZOI at 10µg dose. The Findings indicate that ethanolic plant extracts of *Sida cordifolia* showed antifungal activity.

Discussions

The Total Phenolic content (TPC) of the *Sida cordifolia* seed concentrate was investigated. The gallic acid equivalent in Tamarindus indica is 6.6 mg/g, which is lower than the gallic acid equivalent in *Sida cordifolia* seeds extract [40]. The Total Phenolic content was determined which is 15.76±0.38 mg GAE/g dry extract. The *Sida cordifolia* extract's Total Flavonoid Content (TFC) was also determined which is 8.71±0.65 mg Quercetin equivalent per gram [41]. It was found that 2.3 mg of catechin equivalent per gram of dry extract was the total flavonoid content of *Tamarindus indica* [42], which is somewhat comparable to the *Sida cordifolia* flavonoid's content but the substantial amount of *Sida cordifolia* TPC makes it a potential candidate for formulations with aphrodisiac and fertility ingredients. The fact that the ethanolic extract of *Sida cordifolia* contained more Quercetin equivalents than that of *Dandrophthoe falcata* (0.140 quercetin equivalent/g), suggests that certain aphrodisiac medications contain more flavonoids [43]. Hydroxyl radicals, which are produced when superoxide anion and hydrogen peroxide undergo a reaction sparked by copper or iron, are the most reactive free radicals [44]. Their antioxidant activity is explained by their redox properties [45]. Hydroxyl radicals harm the cell by reacting with proteins, lipids, thiamine, guanosine, polypeptides and polyunsaturated fatty acid moieties [46]. Phenolic compounds attract researchers to concentrate on their organic activities due to their free revolutionary searching and potential cancer prevention agent properties [47]. The ability of phenolic compounds to neutralize antioxidant free radicals that cause oxidative damage to cells is well-known. Due to their beneficial properties, phenolic compounds have attracted a lot of interest in recent microscopy studies [48]. Studies have shown a connection between the antioxidant properties of natural extracts and phenols and flavonoids. Their antioxidant properties are supported by the natural phenolic compounds found in medicinal and edible plants' capacity to donate hydrogen atoms. They are perfect for removing free radicals due to their chemical and structural properties [49]. Due to its capacity to scavenge free radicals promoted by hydroxyl groups, the total phenol concentration can be used as the basis for a quick screening of antioxidant activity [50]. Additionally, the oxidant scavenging activity of plant secondary metabolic products like flavones, flavonoids and condensed tannins depends on the presence of free OH groups, particularly 3-OH [51] [52]. *In-vivo* and *in-vitro* cancer prevention has been demonstrated by plant flavonoids [53]. Due to their redox properties and chemical structures, plants with a high concentration of secondary metabolites, such as flavonoids, carotenoids and

phenolics possess antioxidant activity [54]. Compared to other extracts, ethyl alcoholic extract had a greater impact on DPPH's capacity to scavenge reactive oxygen species at a concentration of 50 g/ml. Hydrogen peroxide (H_2O_2) is an oxidant that can diffuse across biological membranes and is relatively stable and non-radical. However, compared to vitamin C, this effect was much less pronounced at all concentrations. The bioactivity of these crude extracts is due to the higher levels of polyphenols and flavones [55]. Flavonoids are produced by disputing the superoxide anion radical or by reducing molecular oxygen by two electrons, both of which are very effective scavengers of the majority of reactive oxygen species (ROS) [56]. Additionally, it collaborates with substances that can contribute to reducing equivalents to the redevelopment of vitamin E in cell membranes.

The DPPH radical is transformed into the corresponding hydrazine when the research plant extracts react with hydrogen donors. By giving/sharing an electron with the lipid radical, vitamin C transforms into the ascorbate radical [57]. Using an estimate of the concentration required for 50% DPPH radical scavenging, or IC50 (g/ml), it was determined that the alcoholic extracts of the understudied extracts required higher concentrations than the standard for antioxidant activity [58].

At a concentration of 50.0 µg/ml, the ethanolic extract inhibited free radical scavenging by 45.21, free radical scavenging by *Sida cordifolia* Linn. According to DPPH, ABTS, and ferric-reducing antioxidant methods, the alcoholic extract has higher antioxidant activity than commonly used aphrodisiacs [49]. A stable free radical is caused by a calorimetrically measurable change in absorption, which antioxidants can quell by donating a hydrogen atom to, when potassium per-sulfate is used to oxidize ABTS, the pre-formed radical mono cation (ABTS•+) is formed. The concentration of this radical mono-cation decreases when antioxidants that donate hydrogen are present [59]. The focus and response span of the cell reinforcement are taken into consideration when determining its cancer prevention agent movement. Higher concentration of the research drug's alcoholic extracts than the standard ascorbic acid is required for any given level of *antioxidant* activity. At a concentration of 50 µg/ml *Sida cordifolia* extract, the free radical scavenging activity was measured as percentage inhibition was found to be 94.74%. This occurs immediately when the antioxidant activity for scavenging 50% ABTS radicals is measured using IC50 (g/ml). Even at extremely low concentrations, the standard medication Trolox demonstrated superior inhibition to that of the aqueous and alcoholic extracts. Trolox, like vitamin E, is used in biological or biochemical processes as an antioxidant to reduce stress and oxidative damage [60].The total antioxidant assay FRAP, also known as the redox linked colorimetric method, is based on electron transfer.

Trolox is an analog of vitamin E that dissolves in water. The absorbance rises at a predetermined wavelength when an antioxidant reacts with a chromogenic reagent (Fe (II)) [61]. The inhibition rate was found to be 166.67±0.49 percent at concentrations of 40 g/ml. As a result, the ethanolic extract of the research drug consistently displayed greater antioxidant and inhibition activity throughout this investigation. According to the percentage inhibition and IC50 values, ascorbic acid, which has a very high antioxidant capacity in comparison to alcoholic extracts, demonstrated very low IC50 levels [62]. According to the study, this concentrate may be very helpful in the treatment of revolution-related diseases and oxidative damage to male fertility organs.

In Ayurveda and other traditional medical systems, *Sida cordifolia* is one of the most important medicinal plant species used to treat a variety of ailments [63]. The study provides a comprehensive description of the antioxidant and polyphenolic content of *Sida cordifolia* seed extract. It could be argued that the ethanolic seed concentrate of *Sida cordifolia* could be a good source of polyphenolics based on the findings of the current study. Additionally, it demonstrated the highest cell reinforcement movement of any other drug used as antioxidant.

Conclusion

This antioxidant and antimicrobial study revealed that the extract exhibited the most potent free radical scavenging and reducing effect. Phenolic contents have a key role in antioxidant activity, as this study found a linear correlation between antioxidant activity and phenolic contents. Based on the results of *in vivo* studies on biological systems, the search for natural antioxidants, that can be successfully used in subsequent clinical trials, may open new avenues. The study results have created an opportunity for researchers and clinical practitioners to treat oxidative stress-related diseases by natural drugs.

Conflict of Interest:

The authors have no conflict of interest.

Financial Statement:

The corresponding author has managed himself along with the available resources in the Department of Eastern Medicine, GC University Faisalabad.

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Figure 01: Calibration curve of standard GA

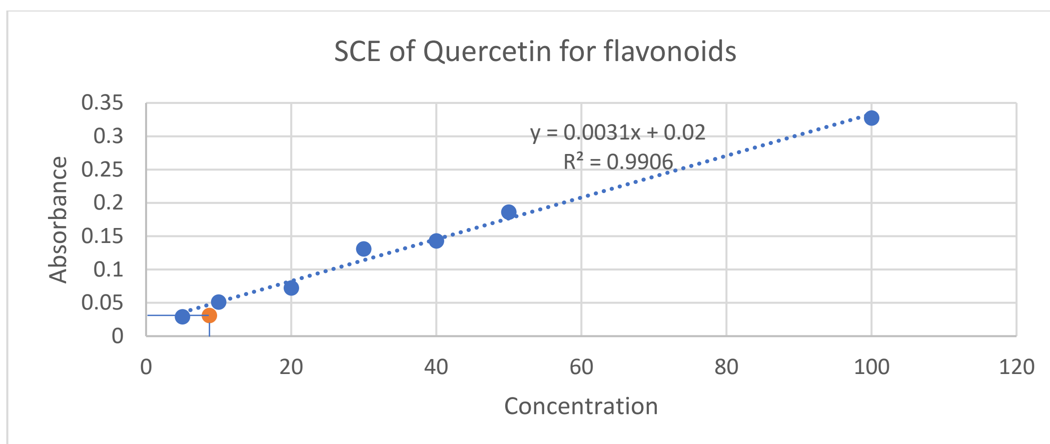

Figure 02: Calibration curve of standard Quercetin

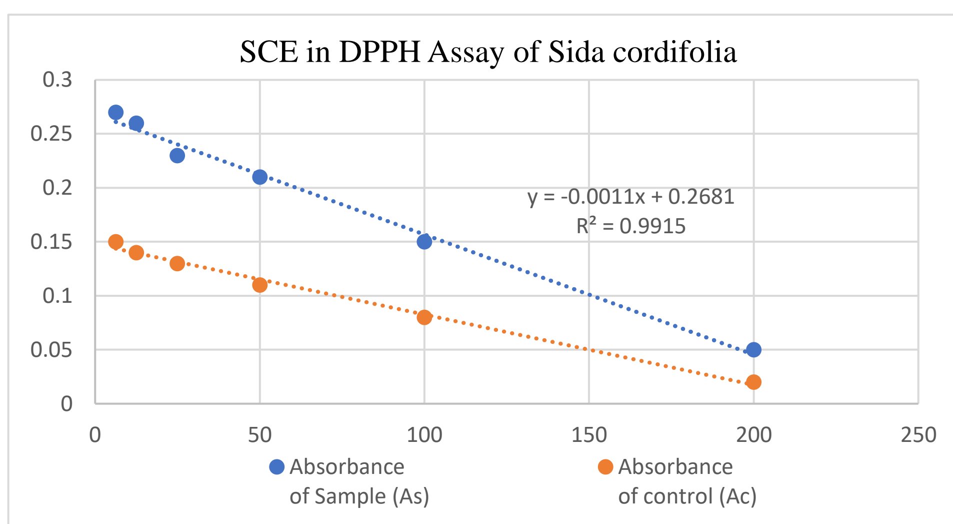

Figure 03: Calibration curve for IC_{50} calculation

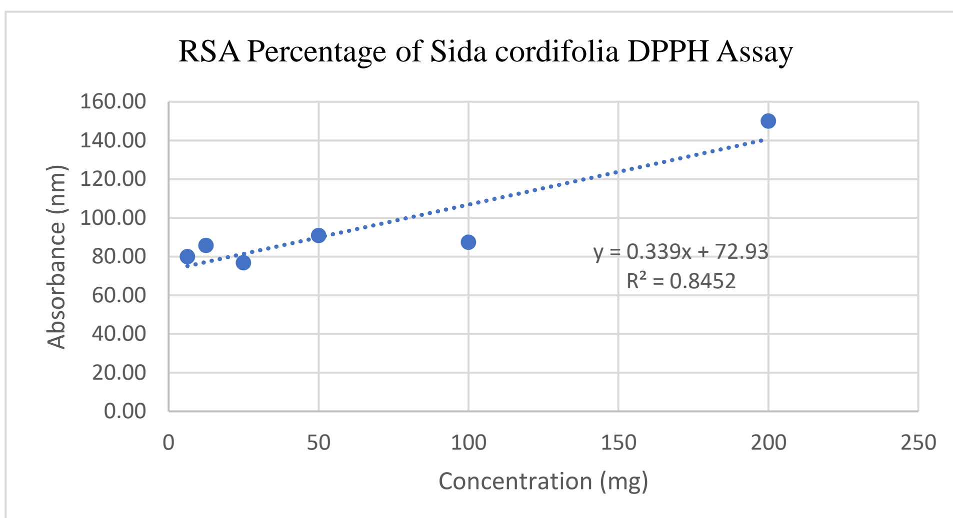

Figure 04: Calibration curve for RSA percentage calculation

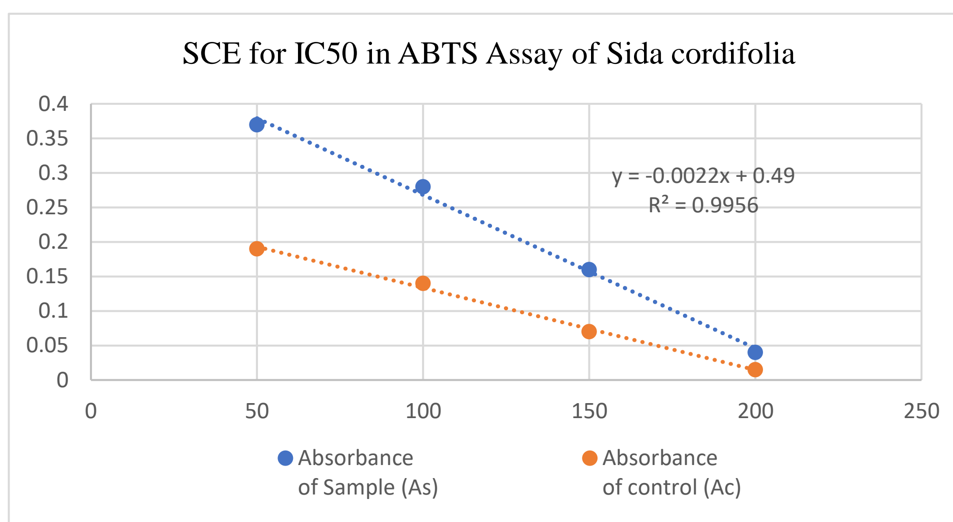

Figure 05: Calibration curve for IC_{50} calculation

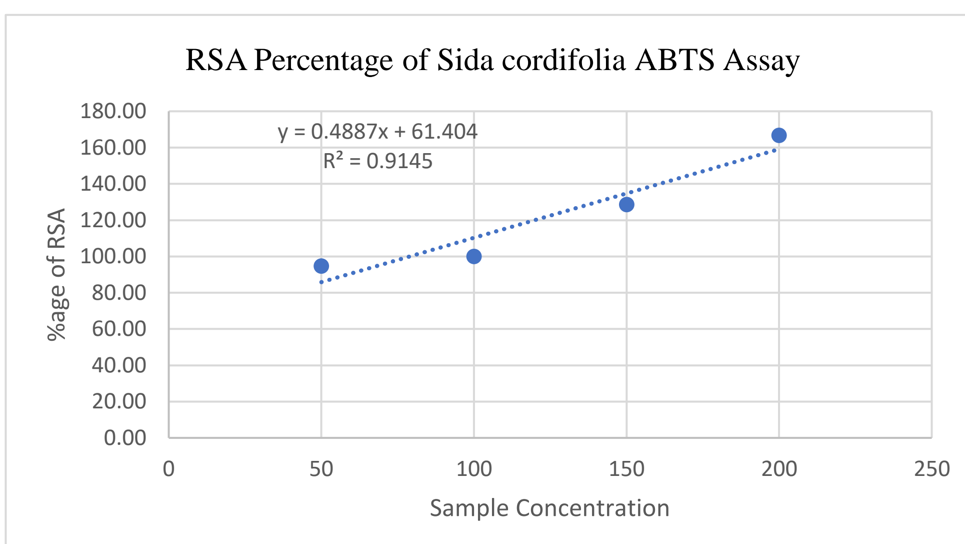

Figure 06: Calibration curve for RSA percentage calculation

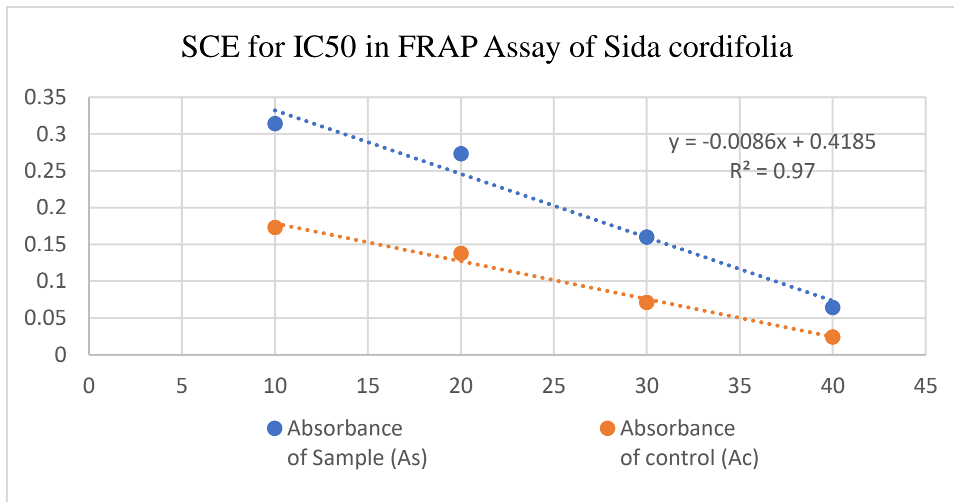

Figure 07: Calibration curve for IC_{50} calculation

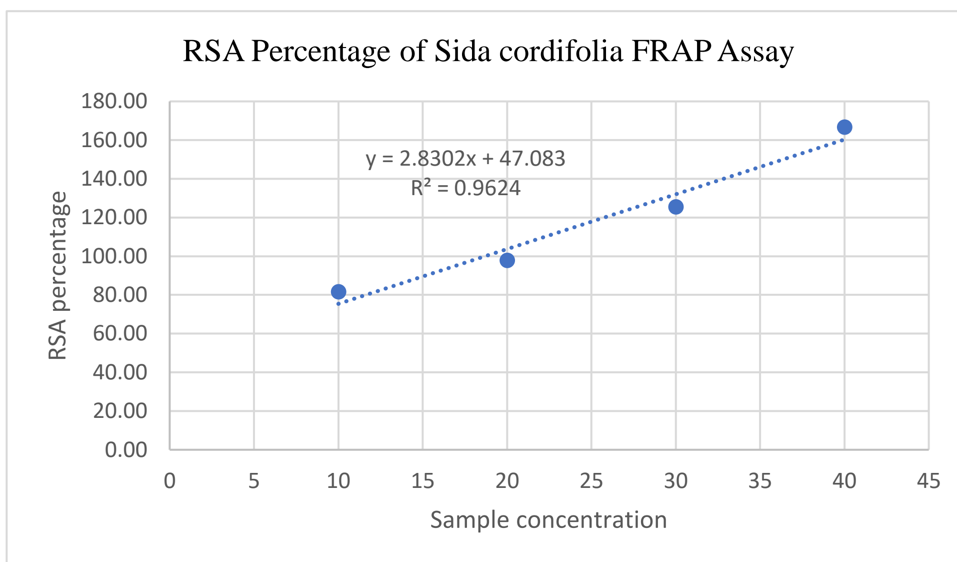

Figure 08: Calibration curve for IC_{50} calculation

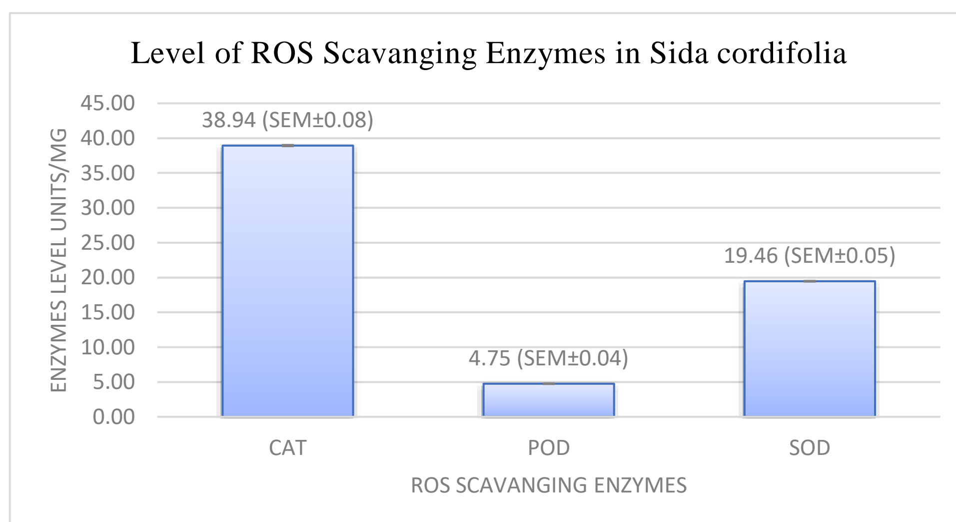

Figure 09: Levels of ROS scavenging enzymes in *Sida cordifolia*

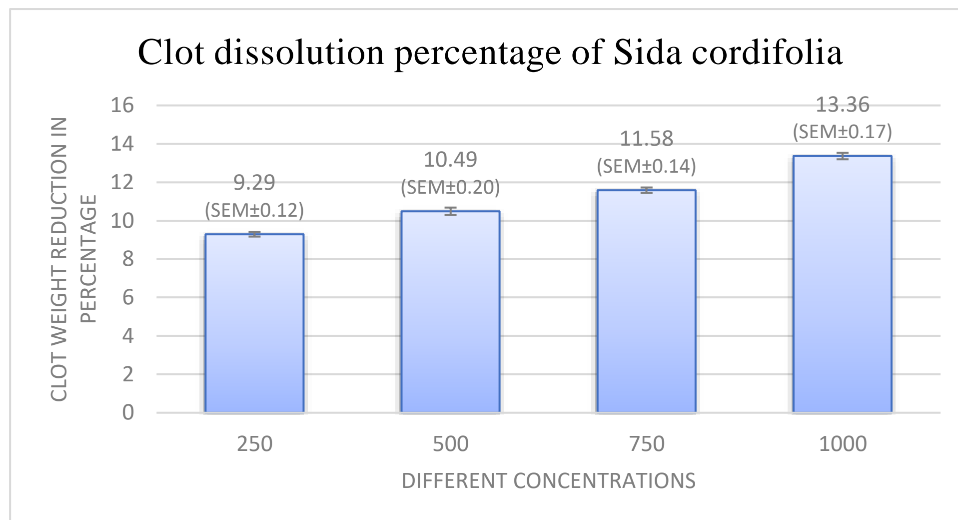

Figure 10: Clot dissolution percentage of *Sida cordifolia*

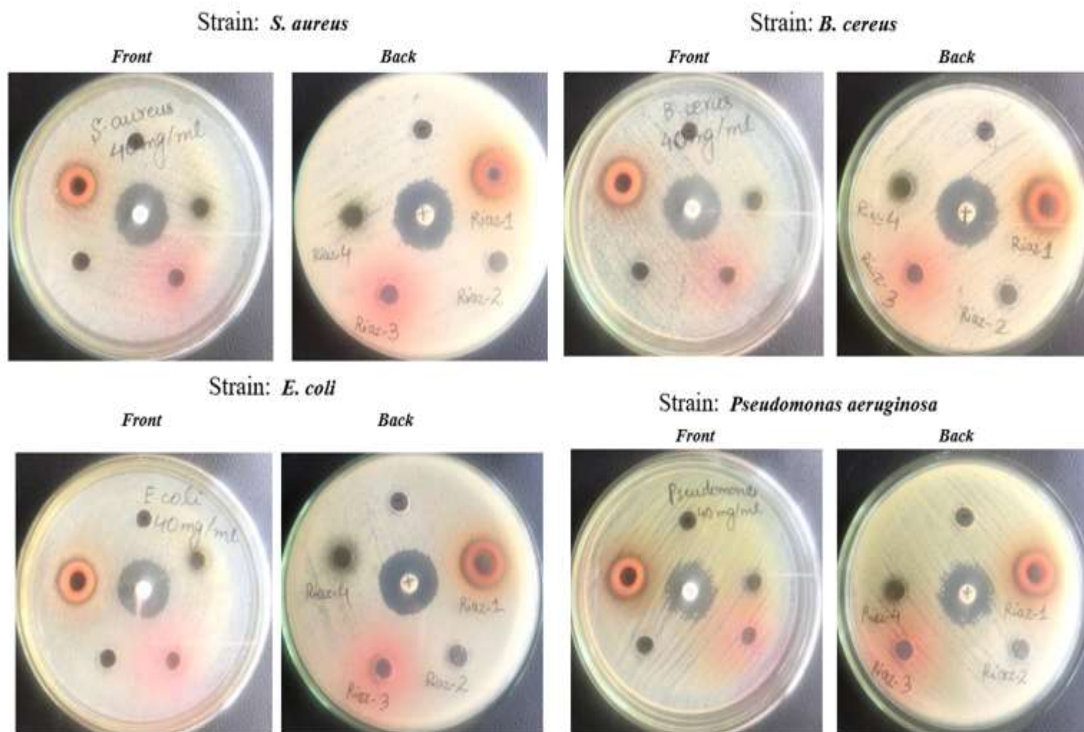

Fig. 11: Zone of inhibition (mm) at the conc. of 40mg of *Sida cordifolia* with antibacterial activity.

Strain: ***Candida albicans***

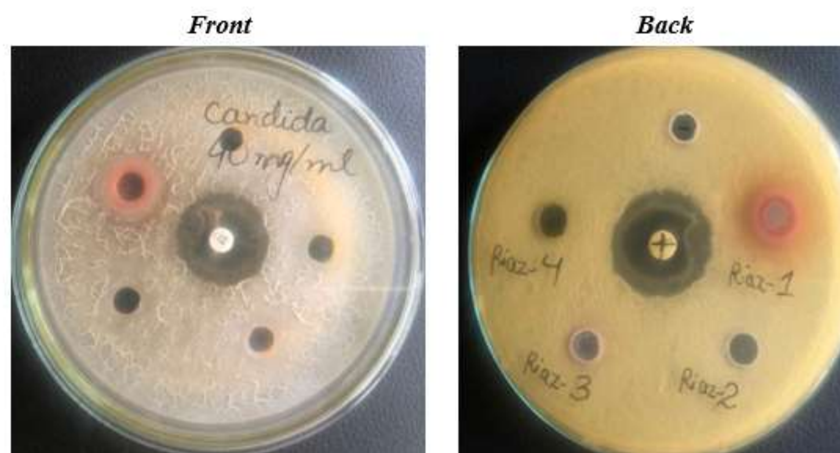

Fig. 12: ZOI produced by *Sida cordifolia* against *Candida albicans*

Table 01: Qualitative Phytochemical Screening of *Sida cordifolia* Seeds Extract

Sr #	Phytochemical	*Sida cordifolia*	Test Name
1	Flavonoids	++++	Shinoda's test
2	Glycosides	+++++	Molisch's test
3	Steroids	+	Liebermann-burchardt test
4	Tannins	++	$FeCl_3$ test
5	Saponins	++	Frothing test
6	Alkaloids	+++++	Dragendorff's test
7	Anthraquinones	++	Borntrager's test

Table: 02 RSA %age, and IC50 values of *Sida cordifolia* in different assays

RSA %age and IC50 value of *Sida cordifolia* in different assays				
	Conc.	RSA %age	SD	IC50
DPPH	50	90.91	± 0.27	10.29
ABTS	50	94.74	± 0.21	22.50
FRAP	40	166.67	± 0.49	33.36

Table: 03 *Sida cordifolia* zones of inhibition in *S. aureus, Bacillus cereus*, *Escherichia coli, P. aeruginosa,* and *Candida albicans* colonies against standards.

Microbial strain	Zone of Inhibition	N	Mean ZOI	S.D	SEM	Sig. (2-tailed)
Staphylococcus aureus	ZOI of Test	5	16.80	0.57	0.25	
	ZOI of Control	5	23.00	0.00	0.00	0.000

Bacillus cereus	ZOI of Test	5	19.80	0.57	0.25	0.000
	ZOI of Control	5	22.00	0.00	0.00	0.000
Escherichia coli	ZOI of Test	5	17.80	0.57	0.25	0.001
	ZOI of Control	5	20.00	0.00	0.00	0.000
Pseudomonas aeruginosa	ZOI of Test	5	17.20	0.57	0.25	0.001
	ZOI of Control	5	22.50	0.00	0.00	0.000
Candida albicans	ZOI of Test	5	15.20	0.57	0.25	0.000
	ZOI of Control	5	25.00	0.00	0.00	0.000